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Application No. 09/787,426

## IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant : Kazutoshi WATANABE et al.

Appl No : 09/787,426

Group Art Unit : 1624

I.A. Filed : September 24, 1999

Examiner : Deepak R. Rao

For : PYRIMIDONE DERIVATIVES

## DECLARATION UNDER 37 C.F.R. § 1.132

Commissioner for Patents  
U.S. Patent and Trademark Office  
Customer Service Window, Mail Stop Amendment  
Randolph Building  
401 Dulany Street  
Alexandria VA 22314

Sir :

I, the undersigned, Dr. Kazutoshi Watanabe, a citizen of Japan, do solemnly declare as follows:

1. That I graduated from the Graduate School of Science (Chemistry), University of Tokyo, receiving a master degree in 1988. I also received a PhD from the Graduate School of Engineering, University of Tokyo, in 2003, an M.S. from the Graduate School of Science (Chemistry), University of Tokyo in 1988, and a B.S. from the Faculty of Science (Chemistry), University of Tokyo in 1986. I have specialized in the field of chemistry and have engaged in research in the chemical field for approximately twenty years. I have been an employee of companies that are associated with Mitsubishi Pharma Corporation since 1988 and am currently employed by Mitsubishi Pharma Corporation in the Pharmaceuticals Research Division.

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2. That the following is a list of articles of which I am a coauthor in the field of art relevant to the above-reference application:

"Antioxidant activity of 3-methyl-1-phenyl-2-pyrazolin-5-one," Yorihiro Yamamoto, Tomohiro Kuwahara, Kazutoshi Watanabe, and Kazuhiko Watanabe, Redox Report 2:333-338 (1996);

"Radical scavenging mechanism of MCI-186," Kazutoshi Watanabe, Kazuhiko Watanabe, and Tetsuo Hayase, Jpn. Pharmacol. Ther. 25(Suppl. 7):189-197 (1997);

"Chemical, pharmacological and clinical profile of a neuroprotective agent edaravone," Kazutoshi Watanabe and Masahiko Tanaka, Pharmacometrics 65:79-88 (2003);

"Research and development of the free radical scavenger edaravone as a neuroprotectant," Toshiaki Watanabe, Masahiko Tanaka, Kazutoshi Watanabe, Yasuo Takamatsu, and Akihiro Tobe, Yakugaku Zasshi 124(3):99-111 (2004);

"Synthesis of the metabolites of a free radical scavenger edaravone (MCI-186, Radicut)," Kazutoshi Watanabe, Masao Taniguchi, and Masaki Shinoda, Redox Report 8(3):157-161 (2003);

"Structure-activity relationship of 3-methyl-1-phenyl-2-pyrazolin-5-one (edaravone)," Kazutoshi Watanabe, Yasuhiro Morinaka, Katsuhiko Iseki, Toshiaki Watanabe, Satoshi Yuki, and Hiroyoshi Nishi, Redox Report 8(3):151-155 (2003);

"Free radical-induced oxidation products of 3-methyl-1-phenyl-2-pyrazolin-5-one (MCI-186)," Kazutoshi Watanabe, Kazuhiko Watanabe, Tomohiro Kuwahara, and Yorihiro Yamamoto, Nihon Yukagakkaishi 46(7):797-801 (1997);

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"Pharmacokinetic studies of 3-methyl-1-phenyl-2-pyrazolin-5-one (MCI-186):

Metabolism in rats, dogs and human," Teiko Komatsu, Hiroshi Nakai, Yasuo Takamatsu, Yasuhiro Morinaka, Kazutoshi Watanabe, Masaki Shinoda, and Seiu Iida, Yakubutsu Dotai 11(5):451-462 (1996);

"Generation of Lithium Enolates Accelerated by Lithium Trifluoromethanesulfonate: Application to the Selective 1,4-Chiral Induction in the Aldol Reaction of *t*-Butyl  $\delta$ -Hydroxy Carboxylates," K. Narasaka, Y. Ukaji, and K. Watanabe, Chem. Lett. 1755-1758 (1986); and "Selective 1,4-Chiral Induction in the Reaction of Enolates Generated from *t*-Butyl  $\delta$ -Hydroxy Carboxylates," K. Narasaka, Y. Ukaji, and K. Watanabe, Bull. Chem. Soc. Jpn. 60:1457-1464 (1987).

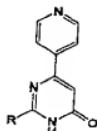
3. That I am one of the inventors of the above-referenced application.
4. That I have reviewed the Office Action mailed June 20, 2006.
5. That experiments have been conducted under my direction to show the inhibitory activity of the compound disclosed in Tani (JP 49035631), as compared to compounds of the present invention recited in Claim 39, against P-GS1 phosphorylation by bovine cerebral TPK1.
6. That the experiments for measuring the inhibitory activity of the compound disclosed in Tani (JP 49035631), as compared to compounds of the present invention, recited in Claim 39, against P-GS1 phosphorylation by bovine cerebral TPK1 were performed as follows:  
A mixture containing 100 mM HEPES-sodium hydroxide (pH 7.2), 1 mM magnesium acetate, 1 mM EGTA, 1 mM dithiothreitol (DTT), 0.02% Tween 20, 7.5  $\mu$ M P-GS1, 10  $\mu$ M [ $\gamma$ -<sup>32</sup>P] ATP (68 kBq/ml), human recombinant TPK1 and compounds shown in the Table below (a

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final mixture contained 1% DMSO deriving from a solution of a test compound prepared in the presence of 10% DMSO) was used as a reaction system. The phosphorylation was started by adding ATP, and the reaction was conducted at 25°C for 2 hours, and then stopped by adding 21% perchloric acid on ice cooling. The reaction mixture was centrifuged at 12,000 rpm for 5 minutes and adsorbed on P81 paper (Whatmann), and then the paper was washed four times with 75 mM phosphoric acid, three times with water and once with acetone. The paper was dried, and the residual radioactivity was measured using a liquid scintillation counter. The results of TPK1 IC<sub>50</sub> values (nM) are shown in the table below with the R group indicated in the table.

7. The compounds of the present invention markedly inhibited the P-GS1 phosphorylation by TPK1, whilst the comparative compound of Tani was revealed to have much lower inhibitory activity.



Tani Compound		IC <sub>50</sub> (nM)	
Present Invention	(CH <sub>3</sub> ) <sub>2</sub> N-	1040.1 nM	
Present Invention	(C <sub>2</sub> H <sub>5</sub> ) <sub>2</sub> N-	369.0 nM	Compound 170
Present Invention	(n-C <sub>3</sub> H <sub>7</sub> ) <sub>2</sub> N-	159.9 nM	Compound 171
Present Invention	C <sub>6</sub> H <sub>5</sub> -CH <sub>2</sub> -NH-	209.0 nM	Compound 397
Present Invention	C <sub>6</sub> H <sub>5</sub> -CH <sub>2</sub> -N(C <sub>2</sub> H <sub>5</sub> ) <sub>2</sub> -	110.3 nM	Compound 404
Present Invention	(i-C <sub>4</sub> H <sub>9</sub> ) <sub>2</sub> N(CH <sub>3</sub> )-	211.0 nM	Compound 440
Present Invention	(cyclo-C <sub>6</sub> H <sub>12</sub> )CH <sub>2</sub> NH-	136.2 nM	Compound 436
Present Invention	C <sub>6</sub> H <sub>5</sub> -NH-	434.5 nM	Compound 168
Present Invention	3-Br-C <sub>6</sub> H <sub>4</sub> -NH-	not tested	Compound 421
Present Invention	3-(OCH <sub>3</sub> )-C <sub>6</sub> H <sub>4</sub> -NH-	139.3 nM	Compound 430
Present Invention	4-(C <sub>2</sub> H <sub>5</sub> )-C <sub>6</sub> H <sub>4</sub> -NH-	250.9 nM	Compound 428

3-Br-C<sub>6</sub>H<sub>5</sub>-; 3-bromophenyl; 3-(OCH<sub>3</sub>)-C<sub>6</sub>H<sub>5</sub>-; 3-methoxyphenyl; 4-(C<sub>2</sub>H<sub>5</sub>)-C<sub>6</sub>H<sub>5</sub>-; 4-ethylphenyl

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8. The undersigned further declares that all statements made herein of his own knowledge are true and that all statements made on information and belief are believed to be true; and further, that these statements were made with knowledge that willful false statements and the like so made are punishable by fine or imprisonment or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the above-captioned application or any patent issuing therefrom.

December 20, 2006  
Date

Kazutoshi Watanabe  
Kazutoshi Watanabe